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which may serve to explain the fact of the existence of indican in urine having so frequently been overlooked.

From the experiments hitherto described, I am justified, I think, in drawing the following conclusions:—

- 1. Human urine contains, under all circumstances, two distinct and peculiar extractive matters, one of which is soluble in alcohol and ether, while the other is soluble in alcohol but insoluble in ether.
- 2. The composition of these bodies is almost always the same, the slight variations which are found to occur being due, not to any difference in the quality or source of the urine employed at various times, but rather to the decomposition which takes place during the process of preparation, and which cannot be entirely avoided.
- 3. Both substances contain nitrogen as an essential constituent, but in so small a proportion as to show that their atomic weight must be very high.
- 4. Both substances have a tendency to take up water, especially when their aqueous solutions are heated or mixed with strong acids.
- 5. The extractive matter insoluble in ether takes up a certain proportion of oxygen, and is converted into a product, which does not differ in its appearance or its most obvious physical properties from the original substance.
- 6. There exists no urinary extractive matter insoluble in alcohol, the substance hitherto so called consisting in most cases of compounds of one of the true extractive matters with various bases.

## III. "On a Crystalline Fatty Acid from Human Urine." By EDWARD SCHUNCK, F.R.S. Received September 21, 1866\*.

The occurrence of fatty matter in urine is a somewhat rare phenomenon, and is generally considered as a symptom of disease, or at least of an abnormal state of the system. In most cases it is found associated with albumen, forming the so-called "chylous urine," in which the fatty matter is suspended in such extremely minute particles as to give the liquid the appearance of milk. In a few instances it has occurred in the shape of fluid oil-globules floating about in the urine; but it is more frequently found enclosed in cells, which sink and form a deposit at the bottom of the vessel. Fatty matter is a constituent of kiesteine, the pellicle which is sometimes formed on the surface of the urine of pregnant women; and a fat resembling butter was obtained from it by Lehmann, though by some authors the very existence of kiesteine as a peculiar deposit is doubted. Lastly, a few cases are described in which a fat-like substance was passed with the urine in the form of small concretions, which, when fresh, were soft and elastic, but dried into hard, yellow, wax-like masses (Heller's

<sup>\*</sup> Read November 15, 1866: see Abstract, vol. xv. p. 258.

urostealith). In no recorded instance was the fatty matter contained in the secretion in a state of true solution.

The accounts which are given of the physical and chemical properties of the fatty matters of urine are extremely vague, and quite insufficient to enable us to identify them, so that it may be concluded that in most cases the quantity obtained was extremely small. Dr. Beale has, indeed, shown that the fatty matter which accumulates in the epithelial cells, passed with the urine in some cases of fatty degeneration of the kidney, contains cholesterine; and Berzelius and Lehmann state that urine, when distilled with the addition of sulphuric acid, yields butyric acid; but in other respects our ignorance is almost complete. None of the works devoted to the subject of urine contain a hint which would lead one to suppose that fatty matter in any form is a constituent of the ordinary healthy secretion.

These few words will probably suffice to give an idea of the present state of our knowledge on this subject from a chemical point of view.

The discovery of which I am about to give an account was a result of the examination of the colouring and extractive matters of urine with which I have been occupied for some time, and which forms the subject of several Papers already communicated to the Royal Society. In the course of my experiments, I observed on several occasions, mixed with the urinary extractive matters, drops of a brown or yellow oil, the appearance of which I could not account for, since it was difficult to conceive how fat of any kind could be deposited from watery solutions of these extractive matters, which generally have an acid reaction; unless, indeed, it was assumed either that it was a product of decomposition, or that the extractive matters possess the property of effecting the solution or suspension of fatty matter in water. On one occasion there was deposited during the evaporation of a watery solution of urian (the extractive matter soluble in ether) a quantity of fatty acid, from which I prepared a baryta-salt soluble in boiling alcohol, and crystallizing from this solution in small scales. Traces of a fat-like substance were almost always obtained on treating watery solutions of the extractive matters with animal charcoal, filtering, treating the charcoal with boiling alcohol, and evaporating the alcoholic liquid. Animal charcoal also effected the separation of a small quantity of fatty matter from urine itself, and this circumstance led me to devise a plan for procuring a quantity sufficiently large to enable me to determine its chief properties. This method I shall now proceed to describe.

Ordinary healthy urine, having been filtered so as to separate all insoluble matter, is passed in successive portions through purified animal charcoal contained in a common percolating apparatus. The percolating liquid appears quite colourless, and devoid of the usual odour of urine. A large quantity of urine may thus be passed with the same effect through a small quantity of charcoal; but at last there arrives a point at which the charcoal, though apparently retaining its decolorizing and deodorizing power undiminished, suffers the liquid to pass through with extreme slowness

only, and the latter, after having percolated, appears rather milky, from a small quantity of white matter suspended in it. At this point it is advisable to discontinue the percolation of urine and to commence washing the charcoal with water. This is continued until every trace of chlorides and phosphates is removed, and the charcoal is then laid to dry, either in the air or at a moderate temperature in a stove. When dry the charcoal is treated with boiling alcohol, to which it communicates a bright yellow colour like that of urine itself, the liquid is filtered, and the process is repeated until the alcohol acquires only a faint yellow colour. To arrive at a point at which it would appear quite colourless seemed to me almost impossible. The whole of the alcoholic liquid, which in any case is considerable in quantity, is now evaporated either spontaneously or at a moderate temperature. The brown syrupy residue which is left on evaporation is mixed with water, which leaves undissolved a quantity of dark-brown semifluid fatty matter to be separated by filtration. The liquid, which has a vellow colour, contains in solution a crystallized organic substance, the occurrence of which in urine has not hitherto been observed. It also contains, provided the evaporation of the alcoholic liquid was conducted spontaneously, a quantity of indican; for on the addition of sulphuric or hydrochloric acid, it deposits flocks of indigo-blue—a reaction which, however, ceases to be produced after the solution has stood for some time in a warm place. Its colour is mainly due to the ordinary extractive matters of urine which it contains.

The fatty matter which is left undissolved by the water has a dark-brown colour and a strongly urinous odour. In order to purify it, it is dissolved in alcohol, and the filtered liquid is evaporated. The residual fatty mass is pressed between blotting-paper, in order to absorb as much as possible the more fluid portion, and it is then redissolved in alcohol. The alcoholic solution is agitated with a little animal charcoal, which deprives it of some of its colour, then filtered and evaporated, when it leaves a brownish-yellow residue, which still retains some of the odour just referred to. By treating it with very dilute spirits this odour, as well as the yellow colour, which seem to belong to the same body, are removed, and an almost white solid fat is left undissolved\*. This may be still further purified by dissolving it in a boiling solution of carbonate of potash. The soap, which separates on cooling, is filtered off, washed with a solution of carbonate of potash, and decomposed with acid. The fatty acid which separates is now quite

\* The filtered alcoholic liquid leaves on evaporation a semifluid, yellow, amorphous fatty matter, having a peculiar urinous odour. When heated on platinum-foil this substance becomes more fluid, gives off a strong smell like that of burning fat, and then burns with a bright flame. It dissolves easily in caustic soda-lye, and the solution froths on being boiled. Its alcoholic solution is yellow, reddens litmus-paper more strongly than the solution of the crystalline acid, and gives with acetate of lead a dirty-yellow flocculent precipitate, which is somewhat soluble in alcohol, since the liquid, when boiled and filtered boiling hot, deposits a quantity of white crystalline grains accompanied by a few thin prismatic crystals.

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colourless. After being washed it is dissolved in alcohol. On spontaneous evaporation the solution leaves a perfectly white crystalline residue consisting of the acid in a state of purity.

As thus prepared, the substance has all the properties characteristic of the group of fatty acids to which palmitic and stearic acid belong. white, has a pearly lustre and a crystalline appearance, and when viewed under the microscope is seen to consist of small star-shaped masses. From a solution in boiling dilute spirits it is deposited, on the solution cooling, in shining scales. The alcoholic solution reddens litmus-paper slightly; it floats on the surface of water, which it repels like all other fats. When the water is heated, it melts into oily drops, which on cooling become solid and crystalline. Its melting-point, as determined with an apparently pure specimen, is 54°.3 C. When impure, i. e. contaminated with the body which imparts the brownish-yellow colour to the crude product, it fuses at a lower temperature. A specimen only slightly coloured melted at 52°.8 C., another at 49°.5 C. When heated between two watch-glasses, the acid fuses and is then volatilized, leaving only a trace of residue, while there is formed on the upper glass an oily sublimate, which on cooling becomes solid and glassy. This sublimate dissolves easily in alcohol, and the solution leaves on spontaneous evaporation a white crystalline residue consisting of needles arranged in star-shaped or feather-like masses. The substance dissolves as easily in ether as in alcohol, and the solution leaves on evaporation a white crystalline mass. It is easily soluble in boiling dilute caustic potash and soda-lye, as well as in aqueous ammonia; these solutions froth on being boiled like those of ordinary soap. The solution in potash deposits on cooling a quantity of white pearly scales, which settle slowly to the bottom of the vessel. The soda compound separates in the form of a thick, white, amorphous soap, a very small quantity of which is sufficient to cause the liquid to gelatinize on cooling. The ammoniacal solution deposits on cooling a quantity of scales, which resemble the potash compound, together with a few crystalline needles. Boiling solutions of carbonate of potash and carbonate of soda also dissolve the acid readily. When the residue left by evaporating the solution in carbonate of potash to dryness is treated with boiling absolute alcohol, an alcoholic solution of the potash-soap is obtained, which, after being filtered from the excess of carbonate of potash and spontaneously evaporated, leaves a residue consisting partly of isolated prismatic crystals, partly of star-shaped The soda compound may in the same manner be obtained in a crystalline state. The alcoholic solution of either of these compounds gives with acetate of baryta a white crystalline deposit. A watery solution gives with nitrate of silver a white, curd-like precipitate, which blackens slowly on exposure to the light. The ammoniacal solution of the acid produces, with the chlorides of barium and calcium, white, flocculent precipitates, which do not become crystalline on standing. The alcoholic solution yields with acetate of lead, an abundant white amorphous precipitate.

These experiments lead to the conclusion that human urine contains in a state of solution a crystalline fatty acid, having the general properties of the members of this class, which are solid at the ordinary temperature. The quantity of this substance which I obtained was too inconsiderable to enable me to determine its composition, and the melting-point therefore afforded the only means of ascertaining whether it is identical with any of the known fatty acids or not. Were it not for the low melting-point there would be nothing to oppose the conclusion that it is palmitic acid, one of the constituents of human fat. It is, however, a well-known fact that mixtures of two solid fatty acids in certain proportions melt at a lower temperature than the most fusible even of the constituents. For instance, according to Heintz, a mixture of 30 parts of stearic acid with 70 of palmitic acid fuses at 55°.1 C., though the melting-point of the former when pure is 70° and of the latter 60°. This urinary acid may therefore be a mixture of this kind and not a peculiar substance—in fact a mixture of the two acids just named, which, according to recent investigations, constitute together what was formerly called margaric acid, the solid acid of human

Considering how many of the organs and secretions of the human body contain fat, it need not excite surprise that a minute quantity of fatty acid should be found in urine also, in consequence of deficient oxidation or from other causes. That it forms a normal constituent of the secretion I do not venture to assert, though the urine employed in my experiments in no case exhibited anything peculiar, and when submitted to the process above described, never failed to yield a little of the fatty acid. The quantity obtained was always extremely small. In one experiment, for instance, 45 litres of urine yielded 0·14 grm. of tolerably pure acid, which, assuming the urine to have been of average composition, would be equal to the 22000th part of its solid constituents. It is far from certain, however, that this was the total quantity contained in it. The simple method of separating the substance from the urine which I have described will enable pathologists to determine whether in cases of disease its quantity is sensibly increased.

The question how this fatty acid, which belongs to a class of bodies almost insoluble in water, comes to be dissolved in urine will naturally suggest itself, but it is one to which it is difficult to find a satisfactory reply. Whether urine is capable of dissolving a small quantity of the acid itself, whether the latter is contained in it in combination with some base, the compound being soluble in water but not decomposable by the weak acids of the urine, or whether, as there seems reason to suspect, the extractive matters promote the solubility of the fatty acid in water, are points on which I express no opinion. That the animal charcoal, when used in the manner above described, effects not a mere filtration, but an actual separation of some of the constituents of urine, may be considered as quite certain.